Advanced glycation end products assessed by skin autofluorescence: a new marker of diabetic foot ulceration

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Advanced Glycation End Products Assessed by Skin Autofluorescence: A New Marker of Diabetic Foot Ulceration

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Abstract

Background: Accumulation of advanced glycation end products (AGEs) may contribute to diabetic foot ulceration (DFU). Our goal was to determine whether AGEs measurement by skin autofluorescence (SAF) would be an additional marker for DFU management.

Patients and Methods: We performed SAF analysis in 66 patients with a history of DFU prospectively included and compared the results with those of 84 control patients with diabetic peripheral neuropathy without DFU. We then assessed the prognostic value of SAF levels on the healing rate in the DFU group.

Results: Mean SAF value was significantly higher in the DFU group in comparison with the control group, even after adjustment for other diabetes complications (3.2 ± 0.6 arbitrary units vs. 2.9 ± 0.6 arbitrary units; $P = 0.001$). In the DFU group, 58 (88%) patients had an active wound at inclusion. The mean DFU duration was 14 ± 13 weeks. The healing rate was 47% after 2 months of appropriate foot care. A trend for a correlation between SAF levels and healing time in DFU subjects was observed but was not statistically significant ($P = 0.06$).

Conclusions: Increased SAF levels are associated with neuropathic foot complications in diabetes. Use of SAF measurement to assess foot vulnerability and to predict DFU events in high-risk patients appears to be promising.

Introduction

Diabetic foot ulceration (DFU) is a worldwide problem leading to a high rate of amputation and functional disability. Recent epidemiological studies have revealed that despite progress in diabetes screening and treatment, DFU remains a significant burden to healthcare systems and society and deeply impairs an individual's life.[1]

Neuropathy exists in 80% of patients with DFU and is clearly the main factor leading to DFU.[2] But, diabetes can also be complicated by a range of biochemical and vascular skin deficits that lead to dermal atrophy and tissue remodeling, limited joint mobility, and pedal edema that, in addition to ill-fitting shoes, may increase the risk of foot complications.[3,4]

Risk of developing ulceration is about 5% after 3 years in patients with diabetes without neuropathy and 14% in those with neuropathy. However, the risk of recurrent ulceration in neuropathic patients with a history of DFU is very much higher and reaches 56% after 3 years.[5,6] The presence of a previous amputation, the duration of diabetes, the hemoglobin A1c (HbA1c) level, or the degree of severity of other chronic complications has not been strongly associated with recurrent ulcer in the literature.[7–9] This gap demonstrates the presence of additional risk factors beyond neuropathy that must be found and taken into account in order to improve current prevention strategies and that might allow for a more effective allocation of the limited resources available for prevention and treatment.

The rationale for assessment of advanced glycation end products (AGEs) through noninvasive techniques for diabetes complications is strong. Indeed, glycation of matrix proteins with accumulation of AGEs is one of the major mechanisms underlying microangiopathy and macroangiopathy.[10] AGEs accumulation leads to basal membrane thickening, inflammation, and oxidative stress, all of which contribute to both skin vulnerability and reduced wound healing capacity.[11,12] Furthermore, the most extensive accumulation of AGEs occurs in tissues that contain proteins with low turnover, such as

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collagen. Accumulation of collagen cross-linking alters the mechanical properties of these tissues with a decrease in elasticity and tensile strength, an increase in mechanical stiffness, and reduced joint mobility, which together can lead to foot ulceration.[13–15]

The noninvasive system used in this study assesses the level of collagen glycation through measurement of skin autofluorescence (SAF) changes.[16] In a previous cohort, SAF was clearly associated with diabetes complications (both microvascular and macrovascular) and also with cardiac mortality in patients with type 2 diabetes.[17–19]

We formulated the hypothesis that evaluation of AGEs by measuring SAF could give additional information to better predict a DFU event and to better assess the potential for healing. Our study therefore has two main goals: (1) to assess whether SAF levels are correlated to DFU status for diabetes patients with peripheral neuropathy and (2) to evaluate whether SAF levels could reflect the healing time in a subgroup of patients with an active DFU at inclusion.

Patients and Methods

Study population

A prospective study was performed in adults with diabetes recruited from diabetes and foot clinics from a hospital-based diabetes center. All patients, in follow-up for neuropathic DFU from January 2011 to December 2011 ($n = 66$) were included after informed consent was obtained. At baseline, 58 patients (88%) had an active DFU, and eight patients (12%) had a prior history of ulceration (DFU group). From the same center 84 patients with similar duration of diabetes and presence of neuropathy but without a history of DFU were included as the control group. Neuropathy was defined, as recommended by the American Diabetes Association[20] to assess DFU risk, by an alteration of monofilament perception and an additional test (absence of ankle reflexes and/or alteration of vibration perception using a 128-Hz tuning fork).

All patients had had a recent evaluation of diabetes complications that included a physical examination, a fundus examination by digital retinography, microalbuminuria and/or proteinuria and creatininemia measurement, and an electrocardiogram. Macroangiopathy was determined by acute vascular events and/or revascularization and/or symptoms of intermittent claudication, absence of peripheral pulses, and/or the presence of abnormal Doppler waveforms in the foot.

Patients with an active DFU at inclusion were followed up for at least 2 months ($n = 58$) after SAF measurement. In the case of an infected wound, the follow-up began after control of the infection. All patients received standard wound care procedure according to by the International Working Group on the Diabetic Foot[21] (i.e., debridement, daily dressing, and off-loading). Healing was defined by complete epidermization of the wound.

SAF measurement

SAF was measured with the AGE Reader (DiagnOptics BV, Gröningen, The Netherlands), as previously described.[16] In brief, the instrument illuminates a 1-cm^2 surface of the skin of the arm with a wavelength of 300–420 nm. Light from the skin is measured with a spectrometer in the 300–600 nm range. In order to assess the interassay variation, the SAF value used for analysis was the average light intensity per nanometer in the 420–600 nm range, divided by the average light intensity per nanometer in the 300–420 nm range, taken from three successive analyses. Results were expressed as the mean of the three consecutive measures in arbitrary units (AU).

In order to exclude the possibility of an increased SAF value caused by a systemic inflammation, measurements were performed in the absence of a clinical wound infection, osteomyelitis, or necrotic tissue. In patients with an active foot ulcer, a second measurement was taken after healing.

Statistical analysis

Continuous variables were described using mean and SD, and categorical variables were described using group size and percentage. DFU patients and control patients were compared using nonparametric tests: the Mann–Whitney test for continuous variables and Fisher's test for categorical variables. In order to study the association between the history of DFU and AGEs measured by SAF, univariate logistic regressions were performed. The model was then adjusted for principal confounders (age, sex, body mass index, diabetes duration, HbA1c level, microvascular disease [retinopathy and/or nephropathy], macrovascular disease [cardiovascular disease and/or lower extremity arterial disease], statin treatment, or angiotensin converting enzyme inhibitors or angiotensin II receptor blockers treatment). Covariates with significant effect in unadjusted analyses were candidates for adjusted regression. Second, the probability of an ulcer healing after 2 months was studied using logistic regression. The same procedure as described for the first goal was applied, restricting the confounder candidates to age, microvascular disease, macrovascular disease, diabetes duration, HbA1c, and DFU duration. Statistical significance was defined as a value of $P \leq 5\%$. Results were expressed as an odds ratio (OR) with a 95% confidence interval (CI). Analyses were performed with R software (version 2.11.1; http://cran.r-project.org/).

Results

Study population characteristics

Patients were more frequently male (68%). The overall mean age was 63.3 ± 11.9 years, and the patients were overweight (body mass index, $29.5 \pm 5.6\ kg/m^2$). Patients had been diagnosed with diabetes for 17 ± 12.4 years, mostly with type 2 diabetes (85%). The mean HbA1c value was $8.1 \pm 1.5\%$ (65 ± 7 mmol/mol) at inclusion. The rate of retinopathy, nephropathy, and macrovascular complications was, respectively, 55%, 50%, and 49%. All patients had an alteration to monofilament perception. Diabetes treatment was insulin (one or more injection) in 73% of cases. The use of statins and angiotensin converting enzyme inhibitors/angiotensin II receptor blockers was, respectively, 67% and 76%.

Risk factors for DFU

SAF was significantly higher in the DFU group than in the control group (3.2 ± 0.6 AU vs. 2.9 ± 0.6 AU) (Fig. 1). A history of DFU was also significantly associated with male gender (77% in the DFU group vs. 60% in the control group) and lower HbA1c ($7.8 \pm 1.4\%$ vs. $8.4 \pm 1.5\%$) (Table 1). Age, duration of diabetes, and the rate of retinopathy, nephropathy, and

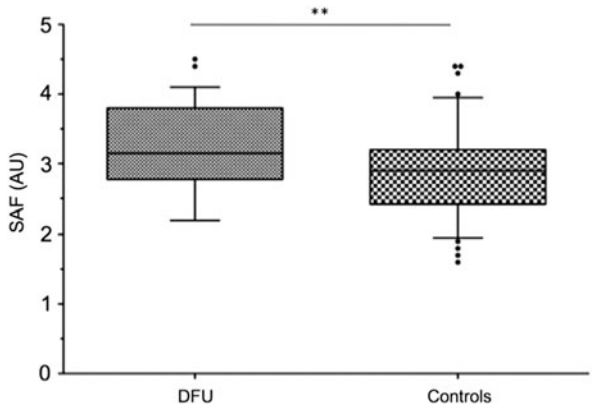

FIG. 1. Skin autofluorescence value (SAF) in patients with diabetic foot ulceration (DFU) ($n=66$) and controls ($n=84$). Data are shown as box and whiskers (5–95th percentile). **$P=0.001$. AU, arbitrary units.

macrovascular disease were not associated with the presence of a history of DFU.

In multivariate analysis SAF was positively correlated with a history of DFU (OR 3.22, 95% CI 1.53–6.79; $P=0.002$). HbA1c showed an inverse correlation (OR 0.76, 95% CI 0.58–0.99; $P=0.046$).

Prognostic value of SAF on DFU healing

The predictive probability of DFU healing was then studied in the 58 patients (88%) with an active wound at baseline. The healing rate after 2 months of appropriate foot care was 46.6% ($n=27$). Univariate analysis resulted in statistically significant positive association between DFU healing and SAF (OR 3.26, 95% CI 1.26–8.46; $P=0.015$), and the duration of DFU (OR 1.06, 95% CI 1.01–1.12; $P=0.028$). In multivariate analysis, although the OR of DFU increased (OR 4.02, 95% CI 0.91–17.85), it was no longer statistically significant ($P=0.067$). Only the duration of DFU remained significantly correlated (OR 1.07, 95% CI 1.01–1.14; $P=0.019$). The SAF value was controlled after complete healing in 37 (74%) patients. For those patients, there was no significant difference in SAF value before and after healing (-0.043 ± 0.210 AU).

Discussion

In this study, we present the first data on SAF values in DFU patients. SAF values are very high, in agreement with the high rate of complications and the high mortality risk of patients with DFU.[22] SAF values significantly correlate with age, duration of diabetes, and microvascular and macrovascular diseases (data not shown). We have found that SAF values were higher in the DFU group than in the control group even after adjustment for other diabetes complications. This result complies with the physiopathological rationale (a recent study show a direct impact of AGEs on skin thickness and vulnerability)[23] and preliminary data on humans.[24] Thus SAF assessment could help to identify a subgroup of neuropathic diabetes patients who are at higher risk of DFU. However, in order to confirm the causal relation between SAF and DFU events, a prospective study on a larger cohort is required.

The link between HbA1c and DFU is still controversial. In the prospective Seattle Diabetes Foot Study, HbA1c was not associated with the risk to develop a DFU.[25] In the EURODIAL Study, an HbA1c level of >7.5% was correlated with a higher risk of DFU recurrence.[26] In our study, HbA1c shows a negative correlation with a history of DFU; HbA1c was lower in the DFU group than in the control group ($7.8\pm1.4\%$ vs. $8.4\pm1.5\%$). Despite the same duration of diabetes, we cannot exclude a sample bias. Indeed, the control group appears to have more insulin deficiency (patients are significantly less treated by oral antidiabetes drugs [23% vs. 32%] and more frequently by insulin [34% vs. 17%]) and so with diabetes is probably more difficult to control than the DFU group. Other confounding factors might exist to explain better glycemic control in the DFU group, such as a modification of dietary strategies, the need for hospitalization, and intensive diabetes care. It is interesting that there is no correlation between SAF and HbA1c.

Table 1. Variables Related to a History of Diabetic Foot Ulceration by Logistic Regression Analysis

	Univariate		*Multivariate*	
Variable	*OR (95% CI)*	P *value*	*OR (95% CI)*	P *value*
SAF	2.63 (1.48–4.66)[a]	0.001[a]	3.22 (1.53–6.79)[a]	0.002[a]
Age (years)	0.99 (0.97–1.02)	0.612	0.97 (0.93–1.00)	0.055
Sex (women)	0.45 (0.22–0.92)[a]	0.029[a]	0.42 (0.17–1.05)	0.063
BMI (kg/m^2)	0.96 (0.90–1.02)	0.200	0.97 (0.90–1.04)	0.345
Macroangiopathy[b]	1.05 (0.55–2.00)	0.885	0.78 (0.33–1.86)	0.575
Retinopathy	1.56 (0.81–3.02)	0.186		
Nephropathy	1.51 (0.79–2.89)	0.213		
Microangiopathy[c]	1.51 (0.75–3.05)	0.251	1.13 (0.46–2.77)	0.784
Diabetes duration	1.01 (0.98–1.04)	0.431	1.01 (0.97–1.05)	0.622
HbA1c	0.74 (0.58–0.94)[a]	0.013[a]	0.76 (0.58–0.99)[a]	0.046[a]
Lipid therapy	1.00 (0.50–1.98)	1.000	1.65 (0.63–4.27)	0.305
BP therapy	1.32 (0.61–2.83)	0.479	0.74 (0.26–2.08)	0.572

[a]Significant values.
[b]Macroangiopathy is the association of cardiovascular disease and lower extremity arterial disease.
[c]Microangiopathy is the association of retinopathy and nephropathy.
BMI, body mass index; BP, blood pressure; CI, confidence interval; HbA1c, hemoglobin A1c; OR, odds ratio; SAF, skin autofluorescence.

Healing time is an important factor in terms of the risk of sepsis and amputation. Some pivotal actors involved in the healing process, such as angiogenesis and keratinocyte migration, are impaired by glycation and tissue accumulation of AGEs.[27–29] It has been reported that blockade of the receptor for AGEs (using the recombinant soluble form of the AGE receptor) restores effective wound closure in mice with diabetes.[30] In the same manner, wound closure is delayed in mice with diabetes exposed to a diet with a high AGEs content versus a low AGEs content.[31] In previous studies, delayed healing has been shown to be characterized by an increase in level of matrix metalloproteinases and a decrease in level of tissue inhibitors of metalloproteinases.[32] Matrix metalloproteinases have been specifically implicated as the major protease family responsible for the degradation of key factors critical to an ulcer's ability to heal.[33] AGEs may play an important role in the impairment of diabetic wound healing by up-regulating matrix metalloproteinase-9 expression in keratinocytes.[34] The correlation between DFU and SAF levels presented in our study is therefore very concordant with these observations.

The question of a possible relationship between SAF and healing is interesting for improvement of patient care. Our results in a subgroup of patients with an active DFU showed a nonsignificant correlation ($P = 0.06$) between SAF and the incidence of healing at 2 months, but the magnitude of effect is rather high. The small number of patients may be the reason for the lack of statistical power. Therefore, the SAF method deserves attention because of its prognostic value for healing.

In conclusion, we have enhanced the relation between SAF results and DFU probability. Athough not highly statistically significant, the value of SAF for DFU healing has equally been shown. Because SAF is very easy to use in everyday clinical practice, further studies should be done to confirm these preliminary data.

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Author Disclosure Statement

No competing financial interests exist.

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